

CLAIMS

What is Claimed is:

- 5 1. A method of providing a therapeutic protein to a customer, said method comprising cloning a nucleic acid encoding said protein into a pCWin1 expression vector as set forth in SEQ ID NO:1, expressing said protein therefrom, and providing said protein to said customer.
- 10 2. A method of providing a therapeutic protein to a customer, said method comprising cloning a nucleic acid encoding said protein into a pCWin2 expression vector as set forth in SEQ ID NO:2, expressing said protein therefrom, and providing said protein to said customer.
- 15 3. A method of providing a therapeutic protein to a customer, said method comprising cloning a nucleic acid encoding said protein into a nucleic acid vector selected from the group consisting of:
- a) a pCWin2/MBP expression vector as set forth in SEQ ID NO:3;
- b) a pCWin2-MBP-SBD (pMS₃₉) expression vector as set forth in
- 20 SEQ ID NO:10; and
- c) a pCWin2-MBP-MCS-SBD (pMXS₃₉) expression vector as set forth in SEQ ID NO:11;
- expressing said protein therefrom, and providing said protein to said customer.
- 25 4. The method of claim 3, wherein said nucleic acid vector comprises a protease cleavage site coding sequence at a location selected from the group consisting of:
- a) between the MBP coding sequence and the therapeutic protein
- 30 coding sequence; and
- b) immediately prior to the start of the C-terminus of the MBP coding sequence.

5 5. The method of claim 2 or 3, wherein said protein is selected from the group consisting of erythropoietin, human growth hormone, granulocyte colony stimulating factor, interferons alpha, -beta, and -gamma, Factor IX, follicle stimulating hormone, interleukin-2, erythropoietin, anti-TNF-alpha, and a lysosomal hydrolase.

10 6. The method of claim 5, wherein said lysosomal hydrolase is selected from the group consisting of beta-glucosidase, alpha-galactosidase-A, beta-hexosaminidase, beta-galactosidase, alpha-galactosidase, alpha-mannosidase, beta-mannosidase, alpha-L-fucosidase, beta-glucuronidase, alpha-glucosidase, alpha-N-acetylgalactosaminidase, and acid phosphatase.

15 7. A method of providing a protein to a customer, said method comprising cloning a nucleic acid encoding said protein into a pCWin1 expression vector as set forth in SEQ ID NO:1, expressing said protein therefrom, and providing said protein to said customer.

20 8. A method of providing a protein to a customer, said method comprising cloning a nucleic acid encoding said protein into a pCWin2 expression vector as set forth in SEQ ID NO:2, expressing said protein therefrom, and providing said protein to said customer.

25 9. A method of providing a protein to a customer, said method comprising cloning a nucleic acid encoding said protein into a nucleic acid vector selected from the group consisting of:

- a) a pCWin2/MBP expression vector as set forth in SEQ ID NO:3;
- b) a pCWin2-MBP-SBD (pMS₃₉) expression vector as set forth in SEQ ID NO:10; and
- c) a pCWin2-MBP-MCS-SBD (pMXS₃₉) expression vector as set

30 forth in SEQ ID NO:11;

 expressing said protein therefrom, and providing said protein to said customer.

10. The method of claim 7, 8 or 9, wherein said protein is selected from the group consisting of a glycosyltransferase and a sugar nucleotide-generating enzyme.

5 11. A method of providing a protein to a customer, said method comprising providing a pCWin1 vector as set forth in SEQ ID NO:1 to a protein production facility, wherein a nucleic acid encoding said protein is cloned into said expression vector and said protein is expressed therefrom in said protein production facility, and providing said protein to said customer.

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12. A method of providing a protein to a customer, said method comprising providing a pCWin2 vector as set forth in SEQ ID NO:2 to a protein production facility, wherein a nucleic acid encoding said protein is cloned into said expression vector and said protein is expressed therefrom in said protein production facility, and providing said protein to said customer.

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13. A method of providing a protein to a customer, said method comprising providing a nucleic acid vector selected from the group consisting of:

a) a pCWin2/MBP expression vector as set forth in SEQ ID NO:3;
20 b) a pCWin2-MBP-SBD (pMS₃₉) expression vector as set forth in SEQ ID NO:10; and

c) a pCWin2-MBP-MCS-SBD (pMXS₃₉) expression vector as set forth in SEQ ID NO:11;

25 to a protein production facility, wherein a nucleic acid encoding said protein is cloned into said expression vector and said protein is expressed therefrom in said protein production facility, and providing said protein to said customer.

14. The method of claim 2, 3, 4, 7, 8 or 9, wherein said method further comprises prior to providing said protein to said customer, at least one
30 glycosyl moiety is added to said protein.

15. The method of claim 14, wherein said glycosyl moiety is added to said protein in vitro.

16. A method of providing a protein to a customer, said method comprising cloning a nucleic acid encoding said protein into nucleic acid vector selected from the group consisting of:

- a) a pCWin1 vector as set forth in SEQ ID NO:1;
- 5 b) a pCWin2 vector as set forth in SEQ ID NO:2;
- c) a pCWin2/MBP vector as set forth in SEQ ID NO:3;
- d) a pCWin2-MBP-SBD (pMS₃₉) vector as set forth in SEQ ID NO:10; and
- 10 e) a pCWin2-MBP-MCS-SBD (pMXS₃₉) vector as set forth in SEQ ID NO:11;

further wherein said method comprises inserting said vector into a bacterial host cell, expressing said protein in said host cell, and providing said protein to said customer.

15 17. The method of claim 16, wherein said method further comprises prior to providing said protein to said customer, at least one glycosyl moiety is added to said protein.

18. The method of claim 16, wherein said glycosyl moiety is
20 added to said protein in vitro.

19. The method of claim 16, wherein said expression vector further comprises an affinity tag coding sequence.

25 20. An isolated pcWIN1 expression vector comprising the sequence set forth in SEQ ID NO:1.

21. An isolated pcWIN1 expression vector consisting of the sequence set forth in SEQ ID NO:1.

30 22. An isolated pcWIN2 expression vector comprising the sequence set forth in SEQ ID NO:2.

23. An isolated pcWIN2 expression vector consisting of the sequence set forth in SEQ ID NO:2.
24. An isolated pcWIN2/MBP expression vector comprising the sequence set forth in SEQ ID NO:3.
25. An isolated pcWIN2/MBP expression vector consisting of the sequence set forth in SEQ ID NO:3.
26. The pcWIN2/MBP expression vector of claim 24, wherein the pCWIN2/MBP vector comprises a protease cleavage site coding sequence adjacent to the MBP coding sequence.
27. An isolated pCWin2-MBP-SBD (pMS₃₉) vector comprising the sequence set forth in SEQ ID NO:10.
28. An isolated pCWin2-MBP-SBD (pMS₃₉) vector consisting of the sequence set forth in SEQ ID NO:10.
29. An isolated pCWin2-MBP-MCS-SBD (pMXS₃₉) vector comprising the sequence set forth in SEQ ID NO:11.
30. An isolated pCWin2-MBP-MCS-SBD (pMXS₃₉) vector consisting of the sequence set forth in SEQ ID NO:11.
31. The pCWin2-MBP-SBD (pMS₃₉) expression vector of claim 27, wherein the pCWin2-MBP-SBD (pMS₃₉) vector comprises a protease cleavage site coding sequence immediately prior to the start of the C-terminus of the MBP coding sequence.
32. A method of expressing a protein, said method comprising cloning a nucleic acid encoding said protein into a pCWin1 expression vector as set forth in SEQ ID NO:1 and expressing said protein therefrom.

33. A method of expressing a protein, said method comprising cloning a nucleic acid encoding said protein into a pCWin2 expression vector as set forth in SEQ ID NO:2 and expressing said protein therefrom.

5 34. A method of expressing a protein, said method comprising cloning a nucleic acid encoding said protein into a nucleic acid vector selected from the group consisting of:

- a) a pCWin2/MBP expression vector as set forth in SEQ ID NO:3;
 - b) a pCWin2-MBP-SBD (pMS₃₉) expression vector as set forth in
10 SEQ ID NO:10; and
 - c) a pCWin2-MBP-MCS-SBD (pMXS₃₉) expression vector as set forth in SEQ ID NO:11;
- and expressing said protein therefrom.

15 35. The method of any one of claims 32-34, wherein said protein is expressed in a prokaryotic cell.